FORM PTO-1390 (Modified) (REV 11-2000) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR
ZIEL1100US	09/890479

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/US00/01923	26 January 2000	27 Janaury 1999

TITLE OF INVENTION
HESPERETIN PRO-FORMS WITH ENHANCED BIOAVAILABLILITY

APPLICANT(S) FOR DO/EO/US
ZIELINSKI LABORATORY

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (24) indicated below.
4. ☐ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☒ has been communicated by the International Bureau.
   c. ☒ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☐ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)). Unexecuted
10. ☐ An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☒ A copy of the International Search Report (PCT/ISA/210).

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☐ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
20. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
21. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
22. ☒ Certificate of Mailing by Express Mail EL617043315US
23. ☒ Other items or information:
    REPLACEMENT SHEET (copy)

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/890479	PCT/US00/01923	ZIEL1100

24. The following fees are submitted:.

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

		CALCULATIONS PTO USE ONLY
☐	Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$1000.00**	
☐	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$860.00**	
☐	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$710.00**	
☐	International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$690.00**	
☒	International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) **$100.00**	
	ENTER APPROPRIATE BASIC FEE AMOUNT =	**$100.00**
	Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).	**$130.00**

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	0 - 20 =	0	x $18.00	**$0.00**
Independent claims	8 - 3 =	5	x $80.00	**$400.00**
Multiple Dependent Claims (**check if applicable**).			☐	**$0.00**

TOTAL OF ABOVE CALCULATIONS =	**$630.00**
☒ Applicant claims small entity status. (See 37 CFR 1.27). The fees indicated above are reduced by 1/2.	**$315.00**
SUBTOTAL =	**$315.00**
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). +	**$0.00**
TOTAL NATIONAL FEE =	**$315.00**
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) (**check if applicable**). ☐	**$0.00**
TOTAL FEES ENCLOSED =	**$315.00**
Amount to be: refunded	$
charged	$

a. ☒ A check in the amount of **$315.00** to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **50-1355** A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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38,347
REGISTRATION NUMBER

July 27, 2001
DATE

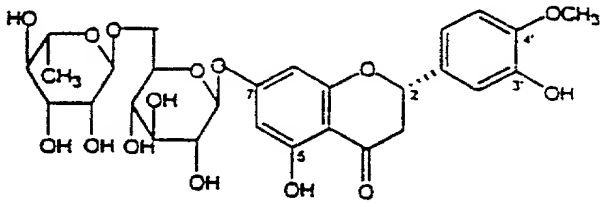
HESPERITIN PRO-FORMS WITH ENHANCED BIOAVAILABILITY

FIELD OF THE INVENTION

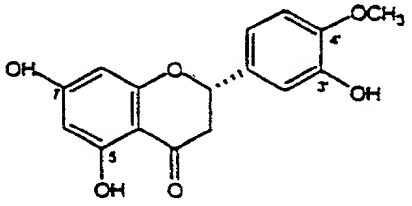
This invention relates generally to the field of bioflavanoids, and more specifically to forms of bioflavanoids that increase their bioavailability, and to the use of these compounds for the treatment of disease.

BACKGROUND OF THE INVENTION

Hesperidin and the a glycone of hesperidin, hesperetin, are flavonoids found in lemons, grapefruits, tangerines, and oranges, and have the following structures:



Hesperidin



Hesperetin

Flavonoids from citrus fruits, known as bioflavonoids exhibit beneficial effects, suppressing oxidative stress, inhibit breast cancer and have anti-inflammatory function.

Hesperidin has been used for the prevention and treatment of cerebral anemia, and pelioma. Recently has been found that hesperidin and hesperetin are effective inhibitors of 3-hydroxy-3-methylglutaryl CoA (U.S. Patent 5, 763,414, herein incorporated by reference). Moreover, hesperetin has topical application for sebum control and treatment of acne in mammalian skin and scalp (U.S. Patent 5,587,176, herein incorporated by reference).

The bioavailability of flavonoids is an important problem in physiological effects, statistically; less than 20% of administered flavonoid is absorbed to blood which subsequently is metabolized to glucuronides and sulfates. Only free flavonoids without sugar molecule, the so-called aglycones are able to pass through the gut wall. Hydrolysis of flavonoid glycosides only occurs in the colon by microorganisms, which in the same time degrade released flavonoids.

SUMMARY OF THE INVENTION

A hesperitin pro-form is provided. The invention provides both a hydrophilic and a lipophilic hesperetin pro-form.

A pharmaceutical composition is provided which is suitable for topical or oral administration in an individual, the composition comprising a hydrophilic hesperetin pro-form and a pharmaceutically acceptable carrier. A pharmaceutical composition is also provided which is suitable for topical or oral administration in an individual, the composition including a lipophilic hesperetin pro-form and a pharmaceutically acceptable carrier.

A method is provided for treating a subject having or at risk of having a cell proliferative disorder, including administering to the subject a therapeutically effective amount of a hesperetin pro-form.

A method is also provided for decreasing oxidative stress in a subject having a disorder associated with oxidative stress, including administering to the subject a therapeutically effective amount of a hesperitin pro-form.

A method is further provided for treating a subject having or at risk of having a disorder associated with sebacous gland activity, including administering to the subject a therapeutically effective amount of a hesperetin pro-form.

A method is provided for treating a subject having or at risk of having a cardiovascular disorder, including administering to the subject a therapeutically effective amount of a hesperetin pro-form.

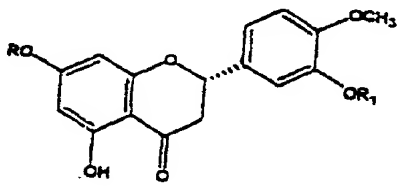
DESCRIPTION OF THE PREFERRED EMBODIMENTS

It must be noted that as used herein and in the appended claims, the singular forms "a," "and," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a cell" includes a plurality of such cells and reference to "the disease" includes reference to one or more diseases and equivalents thereof known to those skilled in the art, and so forth.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this invention belongs. Although any methods, devices and materials similar or equivalent to those described herein can be used in the practice or testing of the invention, the preferred methods, devices and materials are now described.

All publications mentioned herein are incorporated herein by reference in full for the purpose of describing and disclosing the compounds, reagents, and methodologies which are described in the publications which might be used in connection with the presently described invention. The publications discussed above and throughout the text are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior invention.

A "hesperetin pro-form" is hydrophilic or a lipophilic pro-form of hesperetin of the general formula:



A "hydrophilic hesperetin pro-form" is a compound of the formula indicated above wherein:

R is H and R_1 is an organic or an inorganic salt of phosphoric or sulfuric acid residue.

Wherein R_1 is H and R is an organic or an inorganic salt of phosphoric or sulfuric acid residue.

A "lipophilic hesperetin pro-form" is a compound of the formula indicated above, wherein:

One of R and R_1 is a saturated or unsaturated fatty acid moiety.

One of R and R_1 is an acid moiety selected from the group consisting of a straight or branched aliphatic chain, including an alkyl, alkenyl, alkynyl, alkoxyalkyl, alkythioalkyl, aminoalkyl group, including substituted or non-substitued cycloalkyl, and an aromatic group, including aryl, aryalkyl, and substitued derivatives such as where a ring contains one or more nitrogen, sulfer, or oxygens.

Without being bound by theory, a hesperetin pro-form has increased metabolic resistance and improved absorption as compared to hesperetin. "Metabolic resistance" as used herein refers to a decreased ability of an enzyme, normally found in a metabolic pathway, to degrade the compound, as compared to a control compound. "Improved absorption" as used herein refers to an increased ability of an organism to absorb a compound, via any route (*e.g.* dermal absorption, intestinal absorption) as compared to a control compound. Methods and compositions of the present invention provide increased bioavailability of hesperetin by converting this flavanone into pro-compound. This is preferably accomplished by attaching leaving group, which can be readily hydrolyzed under physiologic conditions to produce the starting flavanone.

In one embodiment, hydroxyl groups at 7 and 3' positions are converted to carboxylic, phosphoric and sulfuric acid esters. In an *in vivo* environment, enzymatic or spontaneous hydrolysis of the pro-compounds in gastrointestinal tract or skin, release free hesperetin as a function of time. Kinetics of this process can be controlled by appropriate formulation of the pro-compounds to decrease metabolic transformation and increase absorption of hesperetin in the target cells.

In other aspect of the invention, the pro-compounds may advantageously be employed therapeutically or prophylactically for variety conditions, provided as a dietary supplement, drug or bioactive component of cosmetics.

The molecule of hesperetin has three hydroxyl groups, which differ from each other with different reactivity to acylating reagent. High reactivity of 7-hydroxyl group made possible direct formation of hesperetin-7-esters using activated carboxylic acids or acyl chlorides at the presence of base. Synthesis of hesperetin-3'-eters, required prior esterification of 3'-OH conversion of 7-OH group to benzylformyl or t-butyldimethylsilyl derivatives.

PHARMACEUTICAL COMPOSITIONS

The invention also contemplates various pharmaceutical compositions containing a hesperetin pro-form that are effective in treating a variety of disorders. These disorders include "cell proliferative disorders" , "disorders associated with oxidative stress", "skin disorders", and "cardiovascular disorders".

The term "neoplasia" refers to a disease of inappropriate cell proliferation. This derangement is most evident clinically when tumor tissue bulk compromises the function of vital organs. The term "cell proliferative disorder" denotes malignant as well as nonmalignant cell populations which often appear to differ from the surrounding tissue both morphologically and genotypically. Malignant cells (*i.e.*, tumors or cancer) develop as a result of a multistep process. Concepts describing normal tissue growth are applicable to malignant tissue because normal and malignant tissues can share similar growth characteristics, both at the level of the single cell and at the level of the tissue. Tumors are as much a disease of disordered tissue growth regulation as of disordered cellular growth regulation. The growth characteristics of tumors are such that new cell production exceeds cell death; a neoplastic event tends to produce an increase in the proportion of stem cells undergoing self-renewal and a corresponding decrease in the proportion progressing to maturation (McCulloch, E.A., *et al.*, 1982, "The contribution of blast cell properties to outcome variation in acute myeloblastic leukemia (AML)," *Blood* 59:601-608). In one embodiment, the cells treated by the method of the invention are neoplastic cells.

The term cardiovascular disorder refers to any coronary or cardio-circular disease, including atherosclerosis and hypercholesterolemia.

The term "disorder associated with sebaceous gland activity" refers to a disorder of the pilosebaceous glands of the mammalian skin and scalp. Examples are disorders of sebum secretin such as acne. "Acne" is a pilosebaceous disease characterized by comedo, papules, inflamed nodules and superficial pus-filled cysts. The course and severity of the disease is determined by the interaction between hormones, keratinization, sebum formation and bacteria. The term "treating sebaceous gland activity", as used herein means preventing, retarding, and/or arresting the production of sebum. The tem "treating acne" refers to preventing, retarding, and/or arresting the process of acne formation.

The pharmaceutical compositions according to the invention are prepared by bringing a pro-form of hesperetin of the present invention into a form suitable for administration (e.g., a pharmaceutically acceptable carrier) to a subject using carriers, excipients and additives or auxiliaries. Frequently used carriers or auxiliaries include magnesium carbonate, titanium dioxide, lactose, mannitol and other sugars, talc, milk protein, gelatin, starch, vitamins, cellulose and its derivatives, animal and vegetable oils, polyethylene glycols and solvents, such as sterile water, alcohols, glycerol and polyhydric alcohols. Intravenous vehicles include fluid and nutrient replenishers. Preservatives include antimicrobial, anti-oxidants, chelating agents and inert gases. Other pharmaceutically acceptable carriers include aqueous solutions, non-toxic excipients, including salts, preservatives, buffers and the like, as described, for instance, in Remington's Pharmaceutical Sciences, 15th ed. Easton: Mack Publishing Co., 1405-1412, 1461-1487, 1975, and The National Formulary XIV., 14th ed. Washington: American Pharmaceutical Association, 1975, the contents of which are hereby incorporated by reference. The pH and exact concentration of the various components of the pharmaceutical composition are adjusted according to routine

skills in the art. *See* Goodman and Gilman's The Pharmacological Basis for Therapeutics, 7th ed.

In another embodiment, the invention relates to a method of treating a cell proliferative disorder, a disorder associated with oxidative stress, a skin disorder, and a cardiovascular disorder. These methods involves administering to a subject a therapeutically effective dose of a pharmaceutical composition containing the compounds of the present invention and a pharmaceutically acceptable carrier. "Administering" the pharmaceutical composition of the present invention may be accomplished by any means known to the skilled artisan. By "subject" is meant any mammal, preferably a human.

The pharmaceutical compositions are preferably prepared and administered in dose units. Solid dose units are tablets, capsules and suppositories. For treatment of a patient, depending on activity of the compound, manner of administration, nature and severity of the disorder, age and body weight of the patient, different daily doses are necessary. Under certain circumstances, however, higher or lower daily doses may be appropriate. The administration of the daily dose can be carried out both by single administration in the form of an individual dose unit or else several smaller dose units and also by multiple administration of subdivided doses at specific intervals.

The dosage should not be so large as to cause adverse side effects, such as unwanted cross-reactions, anaphylactic reactions and the like. Generally, the dosage will vary with the age, condition, sex, and extent of the disease in the patient and can be determined by one skilled in the art. The dosage can be adjusted by the individual physician in the event of any contraindications and can be readily ascertained without resort to undue experimentation.

The pharmaceutical compositions according to the invention are in general administered topically, intravenously, orally or parenterally or as implants, but even rectal use is possible in principle. Suitable solid or liquid pharmaceutical preparation forms are, for example, granules, powders, tablets, coated tablets, (micro)capsules, suppositories, syrups, emulsions, suspensions, creams, aerosols, drops or injectable solution in ampule form and also preparations with protracted release of active compounds, in whose preparation excipients and additives and/or auxiliaries such as disintegrants, binders, coating agents, swelling agents, lubricants, flavorings, sweeteners or solubilizers are customarily used as described above. The pharmaceutical compositions are suitable for use in a variety of drug delivery systems. For a brief review of present methods for drug delivery, see Langer, *Science*, 249:1527-1533, 1990, which is incorporated herein by reference.

The pharmaceutical compositions according to the invention may be administered locally or systemically. By "therapeutically effective dose" is meant the quantity of a compound according to the invention necessary to prevent, to cure or at least partially arrest the symptoms of the disease and its complications. Amounts effective for this use will, of course, depend on the severity of the disease and the weight and general state of the patient. Typically, dosages used *in vitro* may provide useful guidance in the amounts useful for *in situ* administration of the pharmaceutical composition, and animal models may be used to determine effective dosages for treatment of particular disorders. Various considerations are described, *e.g.*, in Gilman *et al.*, eds., Goodman and Gilman's: the Pharmacological Bases of Therapeutics, 8th ed., Pergamon Press, 1990; and Remington's Pharmaceutical Sciences, 17th ed., Mack Publishing Co., Easton, Pa., 1990, each of which is herein incorporated by reference. Effectiveness of the dosage can be monitored methods well known to one of ordinary skill in the art.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following examples are illustrative only, and not limiting of the remainder of the disclosure in any way whatsoever.

EXAMPLES

EXAMPLE 1

HESPERETIN-7-PALMITATE.

A solution of palmitoyl chloride (2.1mL, 6.9 mmole) in N,N-dimethylacetamide (3mL) was added dropwise (10 min) to a vigorously stirred suspension containing hesperetin (2g, 6.6 mmole) and Na_2CO_3 (1.1g) in N,N-dimethylacetamide (15mL) at 0°C. The mixture, while stirring, was allowed to reach room temperature and then poured to an ice water. The reaction product was extracted with ethyl acetate (60mL). The organic layer was washed with water, dried over anhydrous Na_2SO_4 and the solvent removed under reduced pressure. The crude product was purified using flash chromatography on silica gel with methylene chloride + chloroform (1 : 1) as the eluent. The final product was crystallized from hexane.

Obtained 2.57g of hesperetin-7-palmitate (72% of the theoretical yield).

^{1}H-NMR (500MHz, DMSO-d_6) δ: 0.848 (t, J=6.8Hz, -CH_2-**CH_3**), 1.230 (broad, 24H, -(**CH_2**)$_{12}$-), 1.607 (m, 2H, -**CH_2**-CH_2-COO-), 2.547 (t, 2H, J=7.3Hz, -**CH_2**-COO-), 2.812 (dd, 1H, J=2.9Hz, J=17.3Hz, -C_3-**H**), 3.360 (dd, 1H, J=12.7Hz, J=17.3Hz, -C_3-**H**), 3.777 (s, 3H, C_4-O**CH_3**), 5.557 (dd, 1H, J=2.9Hz, J=12.7Hz, C_2-**H**), 6.307 (d, 1H, J=1.8Hz, C_6-**H**), 6.320 (d, 1H, J=1.8Hz, C_8-**H**), 6.886 (dd, 1H, J=1.8Hz,

J=8.3Hz, $C_{6'}$-**H**), 6.934 (d, 1H, J=1.8Hz, $C_{2'}$-**H**), 6.944 (d, 1H, J=8.3Hz, $C_{5'}$-**H**), 9.112 (s, 1H, $C_{3'}$-**H**),
11.938 (s, 1H, C_5-O**H**).

Electrospray spectrum showed: m/z 539 [M – H]

EXAMPLE 2

HESPERETIN-3'-STEARATE.

To a vigorously stirred solution of hesperetin (1g, 3.31mmole) in N,N-dimethylacetamide (15mL) containing sodium carbonate (500mg) was added dropwise benzyl chloroformate (0.5mL, 3.5mmole). The reaction mixture was stirred for 15min and then poured to an ice water and extracted with ethyl acetate. The organic phase was washed with water, dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure. The crude product was purified using flash chromatography on silica gel with methylene chloride + chloroform (1 : 1) and crystallized from ethyl acetate/hexane.
Obtained 0.97g of hesperetin-7-benzylcarbonate (67% of the theoretical yield).

A solution of stearoyl chloride (0.85mL, 2.5mmole) in dioxane (2mL) was added dropwise to a stirred solution of hesperetin (1g, 2.29mmole) and diisopropylethylamine (0.45ml, 2.6mmole) in dioxane (10mL). The reaction mixture was stirred for 30min, poured into ice water and extracted with ethyl acetate. The organic layer was washed with 1% HCl, water, dried over anhydrous sodium sulfate and the solvent was removed under reduced pressure. The crude product was chromatographed on silica gel with methylene chloride and chloroform as the eluent. In order to cleave benzylformate group at 7-position, the obtained product was dissolved in ethyl acetate and hydrogenated at the presence of 10% palladium on

charcoal. After filtration and removal of the solvent the residue was crystallized from hexane.

Obtained 0.62g of hesperetin-3'-stearate (48% of the theoretical yield).

^{1}H-NMR (500MHz, DMSO-d_6) δ: 0.848 (t, 3H, J=6.7Hz, -CH_2-CH_3), 1.229 [broad, 28H, -$(CH_2)_{14}$-], 1.633 (m, 2H, -CH_2-CH_2-COO-), 2.549 (t, 2H, J=7.3Hz, -CH_2-COO-), 2.728 (dd, 1H, J=2.8Hz, J=17.0Hz, C_3-**H**), 3.275 (dd, 1H, J=12.7Hz, J=17.0Hz, C_3-**H**), 3.775 (s, 3H, $C_{4'}$-OCH_3), 5.515 (dd, 1H, J=2.8Hz, J=12.7Hz, C_2-**H**), 5.888 (d, 1H, 1.8Hz, C_6-**H**), 5.905 (d, 1H, J=1.8Hz, C_8-**H**), 7.165 (d, 1H, J=8.2Hz, $C_{5'}$-**H**), 7.247 (d, 1H, J=2.0Hz, $C_{2'}$-**H**), 7.376 (dd, 1H, J=2.0Hz, J=8.2Hz, $C_{6'}$-**H**), 11.800 (broad, 1H, C_7-O**H**), 12.124 (s, 1H, C_5-**H**).

Electrospray mass spectrum showed: m/z 567 [M-H].

EXAMPLE 3

HESPERETIN-7-PHOSPHATE AND HESPSRETIN-3'-PHOSPHATE.

To a stirred solution of hesperetin (300mg, 0.99mmole) and phosphorus oxychloride (140µ, 1.5mmole) in dioxane (3mL) was added dropwise (15min) at 0° C a solution of pyridine (120µL, 1.5mmole) in dioxane (1mL). The reaction mixture was stirred for 15min at room temperature, and then water (2mL) was added while stirring was continued for additional 30min. After this time, the mixture was diluted with water, neutralized to pH 5 with $NaHCO_3$ and extracted with ethyl acetate to remove side products and unreacted hesperetin. The aqueous phase was separated, acidified with HCl to pH 2 and extracted three times with ethyl acetate. The extracts were combined, washed with water dried over anhydrous $NaSO_4$, and the solvent removed under reduced pressure.

The crude product was separated using HPLC on Zorbax C8 column (250 x 10mm) with 25% acetonitrile + 75% 0.1M $(NH_4)H_2PO_4$ pH 2.5 adjusted with H_3PO_4 as the mobile phase, and UV detection at 280nm. Obtained two fractions with the retention time 8min and 9min which correspond to hesperetin-7-phosphate and hesperetin-3'-phosphate, respectively. The fractions with the same retention time were pooled, diluted with water and extracted with ethyl acetate. After evaporation of the solvent, the residue was dissolved in ethanol, neutralized with NaOH to pH 5.5 and the solvent removed under reduced pressure.

Obtained: hesperetin-7-phosphate monosodium salt (180mg, 45% yield) and hesperetin-3'-phosphate monosodium salt (50mg, 12% yield)

Spectroscopic data of hesperetin-7-phosphate:

^{1}H-NMR (500MHz, D_2O) δ: 2.783 (dd, 1H, J=2.9Hz, J=17.3Hz, C_3-**H**), 3.174 (dd, 1H, J=12.6Hz, J=17.3Hz, C_3-**H**), 3.845(s, 3H, $C_{4'}$-OC**H**$_3$), 5.394 (dd, 1H, J=2.9Hz, J12.6Hz, C_2-**H**), 6.344 (d, 1H, J=1.8Hz, C_6-**H**), 6.382 (d, 1H, J=1.8Hz, C_8-**H**), 7.021 (s, 2H, $C_{2'}$-**H**, $C_{6'}$-**H**), 7.046 (s, 1H, $C_{5'}$-**H**).

^{31}P-NMR (500MHz, D_2O) δ: -0.5642

Electrospray spectrum showed: m/z 381 [M-H].

Spectroscopic data of hesperetin-3'-phosphate:

^{1}H-NMR (500MHz, D_2O) δ: 2.766 (dd, 1H, J=2.5Hz, 17.3Hz, C_3-**H**), 3.213 (dd, 1H, J=13.3Hz, J=17.3Hz, C_3-**H**), 3.879(s, 3H, $C_{4'}$-O**C**$\mathbf{H}_3$), 5.378 (dd, 1H, J=2.5Hz, J=13.3Hz, C_2-**H**), 5.886 (d, 1H, J=1.8Hz, C_6-**H**), 5.929 (d,1H, J=1.8Hz, C_8-**H**), 7.078 (d, 1H, J=8.4 Hz $C_{5'}$-**H**), 7.153 (d, 1H, J=8.4Hz, $C_{6'}$-**H**), 7.567 (s, 1H, $C_{2'}$-**H**).

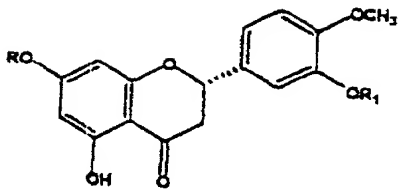
^{31}P-NMR (500MHz, D_2O) δ: 0.4697

Electrospray spectrum showed: m/z 381 [M-H].

Although the invention has been described with reference to the presently preferred embodiment, it should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.

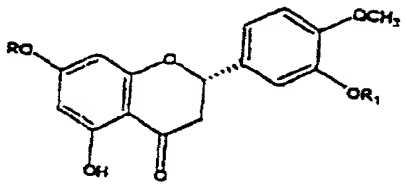
What is claimed is:

1. A hydrophilic hesperitin pro-form of the formula



wherein R is an -H-, and R1 is selected from the group consisting of an organic phosphoric acid salt, an organic sulfuric acid salt, an inorganic phosphoric acid salt and an inorganic sulfuric acid salt, or R1 is an -H- and R is selected from the group consisting of an organic phosphoric acid salt, an organic sulfuric acid salt, an inorganic phosphoric acid salt and an inorganic sulfuric acid salt.

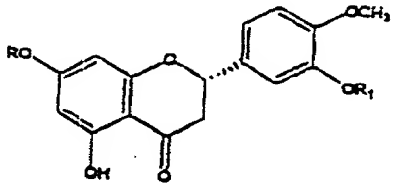
2. A lipophilic hesperitin pro-form of the formula:



wherein R is -H-, and R_1 is selected from the group consisting of a saturated fatty acid moiety, a unsaturated fatty acid moiety, a substituted aliphatic moiety, an aromatic acid moiety, or R_1 is an -H- and R is selected from the group consisting of a saturated fatty acid moiety, a unsaturated fatty acid moiety, a substituted aliphatic moiety, an aromatic acid moiety.

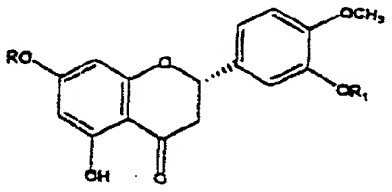
3. The lipophilic hesperitin pro-form of claim 2, wherein said saturated fatty acid moiety, unsaturated fatty acid moiety, substituted aliphatic moiety and aromatic acid moiety comprises from about 1 to about 20 carbons.

4. A pharmaceutical composition suitable for topical or oral administration in an individual, said composition comprising a hydrophilic hesperetin pro-form and a pharmaceutically acceptable carrier, wherein said hesperitin pro-form has the formula:



wherein R is an -H-, and R1 is selected from the group consisting of an organic phosphoric acid salt, an organic sulfuric acid salt, an inorganic phosphoric acid salt and an inorganic sulfuric acid salt, or R1 is an -H- and R is selected from the group consisting of an organic phosphoric acid salt, an organic sulfuric acid salt, an inorganic phosphoric acid salt and an inorganic sulfuric acid salt.

5. A pharmaceutical composition suitable for topical or oral administration in an individual, said composition comprising a lipophilic hesperetin pro-form and a pharmaceutically acceptable carrier, wherein said hesperitin pro-form has the formula:

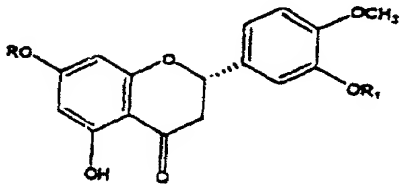


wherein R is -H-, and R1 is selected from the group consisting of a saturated fatty acid moiety, a unsaturated fatty acid moiety, a substituted aliphatic moiety, an aromatic acid moiety, or R1 is an -H- and R is selected from the group consisting of a saturated fatty acid moiety, a unsaturated fatty acid moiety, a substituted aliphatic moiety, an aromatic acid moiety.

6. A method of treating a subject having or at risk of having a cell proliferative disorder, comprising administering to the subject a therapeutically effective amount of a hesperetin pro-form.

7. The method of claim 6, wherein said hesperetin proform is a hydrophilic hesperitin pro-form.

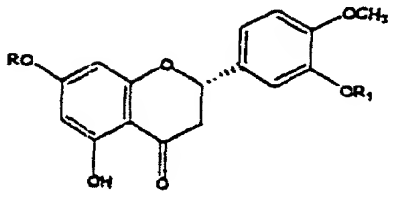
8. The method of claim 7, wherein said hesperetin has the formula



wherein R is an -H-, and R1 is selected from the group consisting of an organic phosphoric acid salt, an organic sulfuric acid salt, an inorganic phosphoric acid salt and an inorganic sulfuric acid salt, or R1 is an -H- and R is selected from the group consisting of an organic phosphoric acid salt, an organic sulfuric acid salt, an inorganic phosphoric acid salt and an inorganic sulfuric acid salt.

9. The method of claim 6, wherein said hesperetin proform is a lipophilic hesperitin pro-form.

10. The method of claim 9, wherein said hesperetin has the formula:



wherein R is -H-, and R1 is selected from the group consisting of a saturated fatty acid moiety, a unsaturated fatty acid moiety, a substituted aliphatic moiety, an aromatic acid moiety, or R1 is an -H- and R is selected from the group consisting of a saturated fatty acid moiety, a unsaturated fatty acid moiety, a substituted aliphatic moiety, an aromatic acid moiety.

11. The method of claim 6, wherein said cell proliferative disorder is selected from the group consisting of breast cancer, skin cancer, uterine cancer, testicular cancer, lung cancer, prostate cancer, liver cancer, and uterine cancer.

12. The method of claim 6, wherein said subject is a human.

13. A method of decreasing oxidative stress in a subject having a disorder associated with oxidative stress, comprising administering to the subject a therapeutically effective amount of a hesperitin pro-form.

14. The method of claim 13, wherein said disorder is selected from the group consisting of diabetes, cerebral anemia, and pelioma.

15. A method of treating a subject having or at risk of having a disorder associated with sebacous gland activity, comprising administering to the subject a therapeutically effective amount of a hesperetin pro-form.

16. The method of claim 14, wherein said disorder associated with sebaceous gland activity is selected from the group consisting of increased sebum production, acne of the skin and acne of the scalp.

17. A method of treating a subject having or at risk of having a cardiovascular disorder, comprising administering to the subject a therapeutically effective amount of a hesperetin pro-form.

18. The method of claim 17, wherein said cardiovascular disorder is selected from the group consisting of atherosclerosis and hypercholesteremia.

ZIEL1100

DECLARATION FOR PATENT APPLICATION

As a below-named inventor, I hereby declare that:

My residence, post office address and citizenship is as stated below next to my name.

I believe that I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled HESPERETIN PRO-FORMS WITH ENHANCED BIOAVAILABILITY , the specification of which

__X__ is attached hereto.

__X__ was filed on July 27, 2001 (Attorney Docket No. ZIEL1100)
as U.S. Application Serial No. _____
and was amended on _____
if applicable (the "Application").

I hereby authorize and request insertion of the application serial number of the Application when officially known.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

COUNTRY	APPLICATION NO.	FILING DATE	PRIORITY CLAIMED	
US	PCT/US00/01923	Janauary 26, 2000	■ Yes	□ No

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of first inventor: Jan E. Zielinski

Inventor's signature: _____

Date: _____

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Citizenship: US

Post Office Address: 820 Sycamore Avenue, #105, Vista, California 92083

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Full name of first inventor: Jan E. Zielinski

Inventor's signature: Jan Zielinski

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